

Activation of NADPH oxidase by purine and pyrimidine nucleotides involves G proteins and is potentiated by chemotactic peptides

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Human neutrophils and HL-60 leukaemic cells possess an NADPH oxidase which catalyses superoxide (O_2^-) formation and is activated by the chemotactic peptide, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMet-Leu-Phe). In dibutyryl cyclic AMP-differentiated HL-60 cells, ATP and UTP in the presence of cytochalasin B activated O_2^- formation with EC_{50} values of $5\ \mu M$ and efficacies amounting to 30 % of that of fMet-Leu-Phe. The potency order of purine nucleotides in activating O_2^- generation was ATP = adenosine 5'-*O*-(3-thiotriphosphate) > ITP > dATP = ADP. Pyrimidine nucleotides activated NADPH oxidase in the potency order UTP > dUTP > CTP = TTP = UDP. Pertussis toxin completely prevented activation of NADPH oxidase by fMet-Leu-Phe and UTP, whereas the effect of ATP was only partially inhibited. ATP and UTP enhanced O_2^- generation induced by fMet-Leu-Phe by up to 8-fold, and primed the cells to respond to non-stimulatory concentrations of fMet-Leu-Phe. Activation of NADPH oxidase by UTP but not by ATP was inhibited by various activators of adenylate cyclase. In dimethyl sulphoxide-differentiated HL-60 cells and in human neutrophils, ATP and UTP *per se* did not activate NADPH oxidase, but they potentiated the effect of fMet-Leu-Phe. Our results suggest that purine and pyrimidine nucleotides act via purino- and novel pyrimidinoceptors respectively, which are coupled to guanine nucleotide-binding proteins leading to the activation of NADPH oxidase. As ATP and UTP are released from cells under physiological and pathological conditions, these nucleotides may play roles as intercellular signal molecules in the activation of O_2^- formation.

INTRODUCTION

Human neutrophils and HL-60 leukaemic cells possess an NADPH oxidase which catalyses superoxide (O_2^-) generation and is activated by the chemotactic peptide, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMet-Leu-Phe) (McPhail & Snyderman, 1984; Rossi, 1986). Formyl peptide receptors interact with guanine nucleotide-binding proteins (G proteins) leading to the activation of phospholipase C, which catalyses the degradation of polyphosphoinositides to inositol phosphates and diacylglycerol (Lad *et al.*, 1985a; Ohta *et al.*, 1985). ADP-ribosylation of the G proteins by pertussis toxin prevents cell activation (Lad *et al.*, 1985a; Ohta *et al.*, 1985; Gierschik *et al.*, 1987; Gilman, 1987). The mechanism by which chemotactic peptides activate NADPH oxidase has been suggested to involve protein kinase C, as cell-permeable diacylglycerol and phorbol myristate acetate are stimulators of protein kinase C and of O_2^- generation (McPhail & Snyderman, 1984; Rossi, 1986). However, we and others have recently provided evidence that activation of NADPH oxidase involves more direct regulation by G proteins (Seifert *et al.*, 1986, 1988; Seifert & Schultz, 1987a,b; Gabig *et al.*, 1987).

Under physiological and pathological conditions, nucleoside triphosphates are released into the extracellular space from a variety of cell types (Goetz *et al.*, 1971; Shirasawa *et al.*, 1983; Gordon, 1986; Hardebo *et al.*, 1987; Forsberg *et al.*, 1987). Released ATP binds

to purinoceptors which regulate many cell functions (Burnstock & Kennedy, 1985; Gordon, 1986). Purinoceptors can be classified into subtypes according to the potency of purinergic agonists. Recently, ATP was reported to activate phospholipase C in HL-60 cells (Dubyak *et al.*, 1987). In addition, ATP induces an increase in the intracellular Ca^{2+} concentration in human neutrophils and enhances fMet-Leu-Phe-induced O_2^- formation (Kuhns *et al.*, 1988). Not only ATP but also UTP plays a role as modulator of cellular functions (Mustard & Packham, 1970; Shirasawa *et al.*, 1983; Dubyak & DeYoung, 1985; Forsberg *et al.*, 1987; Hardebo *et al.*, 1987; Kügelen *et al.*, 1987; Häussinger *et al.*, 1987). There is recent evidence that UTP does not mediate its effects via purinoceptors (Kügelen *et al.*, 1987; Häussinger *et al.*, 1987). These findings prompted us to study the role of purine and pyrimidine nucleotides in the regulation of NADPH oxidase. We report here that ATP and UTP act on purino- and putative pyrimidinoceptors in HL-60 cells and in human neutrophils to activate NADPH oxidase via G proteins.

MATERIALS AND METHODS

Materials

N-t-Butoxycarbonyl-L-phenylalanyl-L-leucyl-L-phenylalanyl-L-leucyl-L-phenylalanine, prostaglandin E_1 , isoproterenol, cholera toxin, 5'-*N*-ethylcarboxamido-adenosine, α,β -methylene-ATP, uridine, uridyl(3'-5')

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uridine, PP_i and TTP were obtained from Sigma Chemie (Deisenhofen, Germany). All other nucleotides were from Boehringer Mannheim (Mannheim, Germany). Pertussis toxin was kindly provided by Dr Motuyuki Yajima (Kyoto, Japan). Sources of other materials and cell culture media have been described elsewhere (Seifert *et al.*, 1986, 1988; Seifert & Schultz, 1987a,b; Seifert & Schächtele, 1988).

Cell culture

HL-60 cells were grown in suspension culture in RPMI-1640 medium supplemented with 10% (v/v) horse serum, 1% (v/v) non-essential amino acids, 2 mM-L-glutamine, 50 U of penicillin/ml and 50 μ g of streptomycin/ml in a humidified atmosphere with 7% CO_2 at 37 °C. To induce differentiation, cells were seeded at a density of 10^6 /ml and were cultured for 48 h in the presence of 0.2 mM-dibutyryl cyclic AMP or for 120 h in the presence of 160 mM-dimethyl sulphoxide (Chaplinski & Niedel, 1982; Seifert & Schächtele, 1988). Cells were harvested by centrifugation for 10 min at 250 g. Wright-Giemsa-stained smears showed that > 95% of the cells were myelocytes or cells beyond this stage.

Preparation of human neutrophils

Heparinized blood was obtained by venous puncture from healthy volunteers of either sex who had taken no drugs for at least 3 weeks. Neutrophils were isolated by dextran sedimentation and centrifugation through Ficoll-Hypaque (Seifert & Schultz, 1987a). Cell preparations contained more than 98% viable neutrophils as judged by Trypan Blue dye exclusion.

Assay for O_2^- generation

NADPH oxidase-catalysed O_2^- generation was monitored by continuous measurement of ferricytochrome C reduction inhibitable by superoxide dismutase,

using a Uvikon 810 dual-beam spectrophotometer (Kontron, Eching, Germany). Reaction mixtures (1 ml) contained 5×10^6 HL-60 cells or 2×10^6 neutrophils, 100 μ M-ferricytochrome C and a buffer consisting of: 138 mM-NaCl, 6 mM-KCl, 1 mM-MgCl₂, 1 mM-CaCl₂, 1 mM-Na₂HPO₄, 5 mM-NaHCO₃, 5.5 mM-glucose and 20 mM-Hepes, pH 7.4. Unless stated otherwise, all assays were performed in the presence of cytochalasin B (1 μ g/ml). Reaction mixtures were preincubated at 37 °C for 3 min. O_2^- generation was initiated by the addition of the stimulus. The V_{max} of O_2^- generation was calculated from the linear sections of absorbance curves (Seifert & Schächtele, 1988).

RESULTS

The effects of various purine nucleotides on NADPH oxidase were studied in HL-60 cells differentiated with dibutyryl cyclic AMP (Fig. 1). In the presence of cytochalasin B, ATP activated NADPH oxidase with an EC_{50} of 5 μ M and a maximum at 100 μ M. The structure of the polyphosphate chain of adenine nucleotides was critical, as adenosine 5'-O-(1-thiotriphosphate), adenylyl imidodiphosphate, α,β -methylene-ATP and β,γ -methylene-ATP did not activate NADPH oxidase at concentrations up to 100 μ M (results not shown). In contrast, adenosine 5'-O-(3-thiotriphosphate) was as potent as ATP. The length of the phosphate chain and the substitution of the ribose moiety were also of importance, as ADP and dATP were far less potent and efficient than ATP. P_i , PP_i , AMP and adenosine did not activate NADPH oxidase at concentrations up to 100 μ M (results not shown). Furthermore, NADPH oxidase activation by purine nucleotides exhibited base specificity. ITP was less potent than ATP, and activated O_2^- generation with an efficacy amounting to 60% of that of ATP. GTP, β,γ -methylene-GTP, guanosine 5'-O-(3-thiotriphosphate)

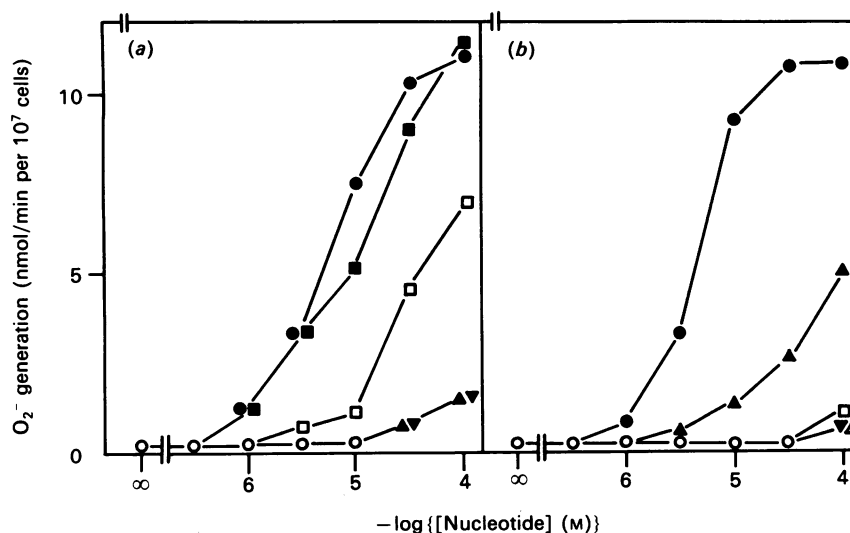


Fig. 1. Activation of NADPH oxidase by purine and pyrimidine nucleotides in HL-60 cells differentiated with dibutyryl cyclic AMP

(a) Concentration-response curves to purine nucleotides: ●, ATP; ■, adenosine 5'-O-(3-thiotriphosphate); ▲, dATP; ▼, ADP; □, ITP. (b) Concentration-response curves to pyrimidine nucleotides: ●, UTP; ▲, dUTP; ▼, UDP; △, TTP; □, CTP. The open circles indicate that nucleotides at the indicated concentrations did not activate O_2^- formation. Nucleotides did not affect cell viability as measured by Trypan Blue dye exclusion and release of lactate dehydrogenase. Data shown represent the means of duplicate assays obtained with one preparation of HL-60 cells, which varied by less than 5%. The variation in O_2^- generation rates in ten experiments carried out with different batches of HL-60 cells was less than 10%.

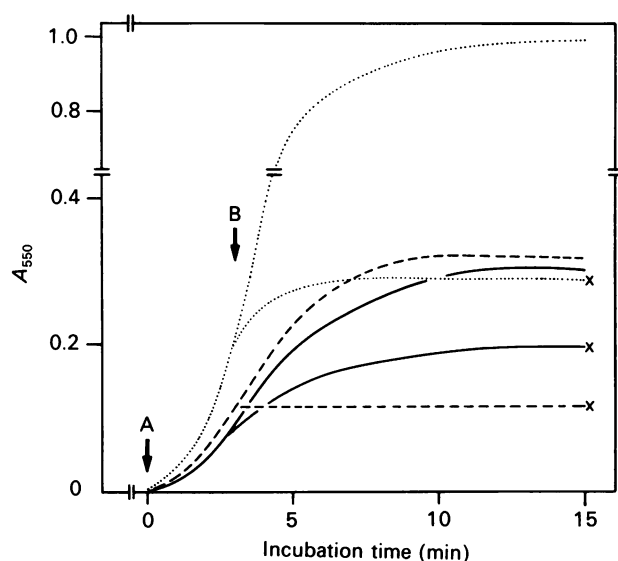


Fig. 2. Reversibility of NADPH oxidase activation in HL-60 cells differentiated with dibutyryl cyclic AMP

A indicates the addition of either $1\ \mu\text{M}$ -fMet-Leu-Phe (\cdots), $100\ \mu\text{M}$ -ATP ($---$) or $100\ \mu\text{M}$ -UTP ($—$). B marks the addition of *N*-t-butoxycarbonyl-L-phenylalanyl-L-leucyl-L-phenylalanyl-L-leucyl-L-phenylalanine ($10\ \mu\text{M}$) to assays containing fMet-Leu-Phe ($\cdots \times$) or the addition of hexokinase (20 units) to assays containing ATP ($--- \times$) or UTP ($— \times$). Superimposed typical tracings of parallel experiments are shown. Similar results were obtained in four experiments carried out with different preparations of HL-60 cells.

and guanylyl imidodiphosphate at concentrations up to $100\ \mu\text{M}$ were inactive (results not shown). Among the pyrimidine nucleotides, UTP activated NADPH oxidase with a potency and an efficacy comparable to that of ATP. dUTP, TTP, CTP and UDP were far less potent and efficient activators of NADPH oxidase than was UTP. UMP, uridine, uridyl(3'-5')uridine and UDP-glucose were inactive at concentrations up to $100\ \mu\text{M}$ (results not shown). Recently, α,β -methylene-ATP has been reported to be a partial antagonist at certain purinoceptors (Katsuragi & Furukawa, 1985). However, α,β -methylene-ATP at a concentration of $100\ \mu\text{M}$ did not prevent activation of O_2^- formation by ATP or UTP at a concentration of $10\ \mu\text{M}$ (results not shown). Similar results as with α,β -methylene-ATP were obtained with AMP and UMP.

Some characteristics of NADPH oxidase activation by UTP, ATP and fMet-Leu-Phe were compared (Fig. 2). The efficacies of maximally stimulatory concentrations of ATP and UTP to activate O_2^- generation amounted to 30–50% of that of a maximally effective concentration of fMet-Leu-Phe, with respect both to V_{max} and to the absolute amount of O_2^- generated. In addition, nucleotides and chemotactic peptides reversibly activated O_2^- formation which ceased after 8–15 min. In agreement with a recent report (Korchak *et al.*, 1984), NADPH oxidase activation by fMet-Leu-Phe was rapidly terminated upon addition of a 10-fold excess of its competitive antagonist, *N*-t-butoxycarbonyl-L-phenylalanyl-L-leucyl-L-phenylalanyl-L-leucyl-L-phenylalanine, suggesting that the continuous occupation

of formyl peptide receptors by agonist is required to maintain O_2^- formation. As no competitive antagonists for ATP and UTP were available, we used hexokinase reactions to remove the nucleotides from the reaction mixtures. Hexokinase catalyses the phosphorylation of glucose and, thereby, the dephosphorylation of ATP to ADP. Yeast hexokinase rapidly terminated O_2^- generation induced by ATP and gradually inhibited that induced by UTP, corresponding to the findings that ATP is a much better substrate for yeast hexokinase than is UTP (Colowick, 1973), and that both ADP and UDP are not efficient activators of O_2^- formation. Thus, the continuous presence of ATP and UTP was essential to maintain O_2^- formation. In addition to reversibility, activation of NADPH oxidase by fMet-Leu-Phe and nucleotides depended on the presence of extracellular Ca^{2+} ($1\ \text{mM}$). In the absence of free Ca^{2+} , these stimuli did not activate O_2^- formation. Mg^{2+} ($1\ \text{mM}$) was no substitute for Ca^{2+} and did not enhance O_2^- formation in the presence of Ca^{2+} (results not shown).

In order to elucidate the role of G proteins in NADPH oxidase activation by extracellular nucleotides, HL-60 cells were treated with pertussis toxin. Pertussis toxin inhibited in a concentration-dependent fashion, activation of NADPH oxidase by fMet-Leu-Phe, ATP and UTP but not by phorbol myristate acetate, which circumvents receptor stimulation by directly activating protein kinase C (Fig. 3). The inhibitory effect of pertussis toxin on the fMet-Leu-Phe- and UTP-induced O_2^- generations was half-maximal at $10\ \text{ng/ml}$ and was maximal at $100\ \text{ng/ml}$. In contrast to its effects on NADPH oxidase activation by fMet-Leu-Phe and UTP, pertussis toxin inhibited the ATP-induced O_2^- generation only by up to 60%. The effect of pertussis toxin on O_2^- formation was time-dependent. Half-maximal inhibition of O_2^- generation was observed after 30–45 min of toxin treatment. Inhibition of the fMet-Leu-Phe- and UTP-induced O_2^- generation was complete after an incubation time of 2 h. In contrast, treatment of the cells with pertussis toxin for up to 3 h did not completely suppress the stimulatory effect of ATP. These results indicate that UTP and ATP did not activate NADPH oxidase by similar mechanisms.

The repeated exposure of human neutrophils to hormonal agonists desensitizes the cells to generating O_2^- upon restimulation (Smith *et al.*, 1984). Therefore, desensitization experiments were performed in HL-60 cells. Cells were pretreated with fMet-Leu-Phe, ATP or UTP at maximally stimulatory concentrations, and O_2^- generation upon re-exposure to these agents was measured (Table 1). Pretreatment of the cells with fMet-Leu-Phe desensitized O_2^- generation upon re-exposure to the chemotactic peptide but not to ATP or UTP. Conversely, pretreatment with ATP or UTP did not desensitize the cells towards fMet-Leu-Phe but did desensitize them towards both ATP and UTP, a phenomenon known as heterologous desensitization (Smith *et al.*, 1984).

These results prompted us to study the interaction of chemotactic peptides and nucleotides on O_2^- formation in HL-60 cells differentiated with dibutyryl cyclic AMP or dimethyl sulphoxide and in human neutrophils. The experiments depicted in Fig. 4 were carried out in the absence of cytochalasin B, which enhances fMet-Leu-Phe-induced O_2^- generation by inhibiting receptor sequestration and by expressing new receptors (Jesaitis

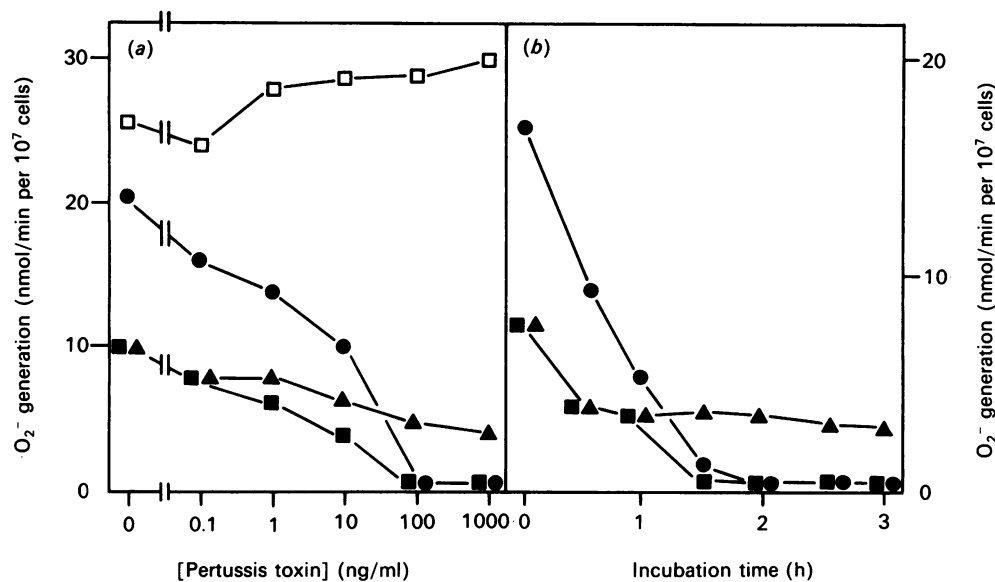


Fig. 3. Inhibition of O_2^- formation by pertussis toxin in HL-60 cells differentiated with dibutyryl cyclic AMP

HL-60 cells were suspended in culture medium at a concentration of 2×10^6 cells/ml and incubated in the presence of pertussis toxin or its carrier. Cell viability after incubation with pertussis toxin was $> 95\%$ as revealed by Trypan Blue dye exclusion. Following toxin treatment, cells were harvested and assayed for O_2^- formation. (a) Concentration-dependence. Cells were incubated for 3 h in the presence of the indicated concentrations of toxin. (b) Time-dependence. Cells were incubated in the presence of pertussis toxin (100 ng/ml) for the indicated periods of time. The concentrations of stimuli for determination of O_2^- generation were as follows: □, 100 ng of phorbol myristate acetate/ml; ●, 1 μ M-fMet-Leu-Phe; ▲, 100 μ M-ATP; ■, 100 μ M-UTP. Data shown represent means of assay duplicates obtained with one batch of HL-60 cells which varied by less than 5%. Similar results were obtained in three experiments carried out with different preparations of HL-60 cells which varied by less than 10%.

et al., 1986). In the absence of cytochalasin B, dibutyryl cyclic AMP-differentiated HL-60 cells generated O_2^- at rates amounting to 30–60% of those in the presence of cytochalasin B upon exposure to UTP (100 μ M) and fMet-Leu-Phe (1 μ M) (see Figs. 1, 3 and 4). UTP and ATP at concentrations of up to 10 μ M did not activate NADPH oxidase in the absence of cytochalasin B. UTP

(0.1–10 μ M) enhanced in a concentration-dependent manner O_2^- generation induced by fMet-Leu-Phe at submaximally and maximally stimulatory concentrations by up to 8-fold. In addition, UTP made the cells responsive to a non-stimulatory concentration of fMet-Leu-Phe (1 nM), a process called priming (McPhail & Snyderman, 1984; Rossi, 1986). Similar results were obtained with ATP as with UTP. Upon exposure to fMet-Leu-Phe, dimethyl sulphoxide-differentiated HL-60 cells generated O_2^- at much lower rates than dibutyryl cyclic AMP-differentiated cells (Table 2). This finding is in agreement with a previous report showing that dibutyryl cyclic AMP-differentiated cells expressed much higher numbers of formyl peptide receptors than dimethyl sulphoxide-differentiated cells (Chaplinski & Nidel, 1982). In dimethyl sulphoxide-differentiated HL-60 cells, nucleotides at concentrations up to 100 μ M did not activate NADPH oxidase. However, ATP and UTP potentiated fMet-Leu-Phe-induced O_2^- generation. By analogy to dimethyl sulphoxide-differentiated HL-60 cells, nucleotides *per se* failed to activate NADPH oxidase in human neutrophils. In the absence of cytochalasin B, UTP enhanced the effect of fMet-Leu-Phe in neutrophils by 3-fold, whereas in the presence of cytochalasin B, UTP stimulated O_2^- formation only by 42%.

In human neutrophils, the effects of fMet-Leu-Phe are inhibited by activators of adenylate cyclase, such as 5'-N-ethylcarboxamidoadenosine, isoproterenol, prostaglandin E_1 and cholera toxin (Bokoch & Gilman, 1984; Lad *et al.*, 1985b; Cronstein *et al.*, 1985; Gryglewski *et al.*, 1987). Furthermore, these agents differentially inhibit O_2^- formation induced by different classes of

Table 1. Desensitization of O_2^- generation in HL-60 cells differentiated with dibutyryl cyclic AMP

HL-60 cells were harvested, suspended at a density of 2×10^7 cells/ml in buffer for determination of O_2^- generation and incubated for 10 min in the presence of buffer (control), fMet-Leu-Phe, ATP or UTP at the indicated concentrations at 37 °C. Thereafter, stimuli were diluted by suspending the cells in a 10-fold excess of volume of buffer not supplemented with $CaCl_2$ and stimulus. Cells were harvested and assayed for O_2^- generation with various stimuli. The means of three experiments performed with different preparations of HL-60 cells are given; these varied by less than 10%.

| Treatment | O_2^- generation (% of control) | | |
|--------------------------|---------------------------------------|-------------------|-------------------|
| | Stimulus ... fMet-Leu-Phe (1 μ M) | ATP (100 μ M) | UTP (100 μ M) |
| fMet-Leu-Phe (1 μ M) | 20 | 106 | 93 |
| ATP (100 μ M) | 93 | 42 | 20 |
| UTP (100 μ M) | 101 | 18 | 26 |

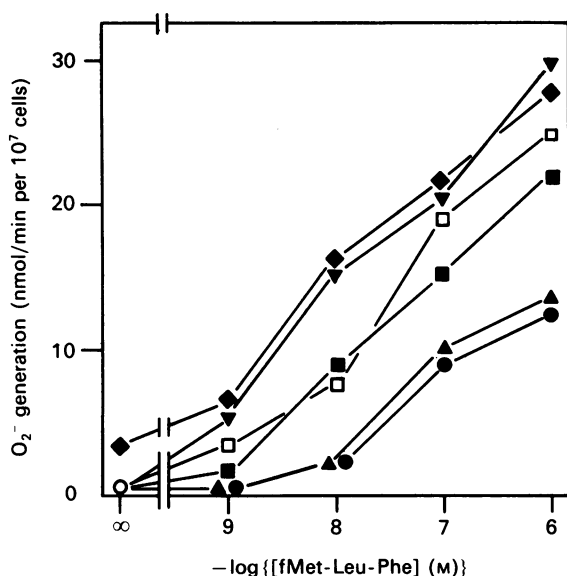


Fig. 4. Synergistic activation of NADPH oxidase by nucleotides and chemotactic peptides in HL-60 cells differentiated with dibutyryl cyclic AMP

The effects of various concentrations of fMet-Leu-Phe were studied in the presence of one fixed concentration of ATP and four fixed concentrations of UTP. Nucleotides and fMet-Leu-Phe were added simultaneously to reaction mixtures. ●, Buffer (control); □, 10 μ M-ATP; ▲, 0.1 μ M-UTP; ■, 1 μ M-UTP; ▼, 10 μ M-UTP; ◆, 100 μ M-UTP. Assays were carried out in the absence of cytochalasin B. Data shown represent the means of assay duplicates obtained with one preparation of HL-60 cells, which varied by less than 5%. Similar results were obtained in five experiments carried out with different batches of HL-60 cells, which varied by less than 10%.

Table 2. Synergistic activation of NADPH oxidase by nucleotides and chemotactic peptides in HL-60 cells differentiated with dimethyl sulphoxide and in human neutrophils: effect of cytochalasin B

Activation of O_2^- formation by fMet-Leu-Phe, ATP, UTP and combinations of these stimuli as well as the interaction with cytochalasin B (CB) were studied in dimethyl sulphoxide-differentiated HL-60 cells and in human neutrophils. The concentrations of stimuli were 1 μ M (fMet-Leu-Phe), 10 μ M (ATP and UTP) and 1 μ g/ml (CB). The means \pm S.E.M. of assay triplicates are shown. Similar results were obtained with three batches of HL-60 cells and with neutrophils from five different donors which varied less than 10%. n.d., not done.

| Stimulus | Cell line... | O_2^- generation (nmol/min per 10^7 cells) | |
|-------------------------|--------------|---|-------------------|
| | | HL-60 cells | Human neutrophils |
| fMet-Leu-Phe | | 2.7 \pm 0.3 | 5.3 \pm 0.2 |
| ATP | | 0 | 0 |
| UTP | | 0 | 0 |
| fMet-Leu-Phe + ATP | | 5.0 \pm 0.4 | 15.3 \pm 1.0 |
| fMet-Leu-Phe + UTP | | 5.1 \pm 0.2 | 15.8 \pm 0.8 |
| fMet-Leu-Phe + CB | | n.d. | 32.0 \pm 1.6 |
| UTP | | 0 | 0 |
| fMet-Leu-Phe + UTP + CB | | n.d. | 45.5 \pm 4.0 |

Table 3. Inhibition of O_2^- formation by various activators of adenylate cyclase in HL-60 cells differentiated with dibutyryl cyclic AMP

HL-60 cells were cultured for 48 h in the presence of 0.2 mM-dibutyryl cyclic AMP to induce expression of formyl peptide and nucleotide receptors. Thereafter, cells were harvested and suspended in the buffer for O_2^- generation containing no dibutyryl cyclic AMP. The activators of adenylate cyclase, 5'-N-ethylcarboxamido-adenosine (NECA), isoproterenol, prostaglandin E_1 or buffer (control) were added to reaction mixtures at the indicated concentrations 3 min prior to fMet-Leu-Phe, ATP or UTP. Cells were treated with cholera toxin or its carrier (control) for 2 h according to the procedure described in the legend to Fig. 3. Data shown represent the means of four to seven experiments carried out with different preparations of HL-60 cells which varied less than 10%.

| Treatment | O_2^- generation (% of control) | | |
|----------------------------------|--|----------------------|----------------------|
| | Stimulus ... fMet-Leu-Phe (1 μ M) | ATP (100 μ M) | UTP (100 μ M) |
| NECA (10 μ M) | 66 | 85 | 53 |
| Isoproterenol (1 μ M) | 90 | 98 | 76 |
| Prostaglandin E_1 (10 μ M) | 89 | 92 | 55 |
| Cholera toxin (1 μ g/ml) | 36 | 92 | 30 |

hormone agonist. In preliminary experiments, the concentrations of adenylate cyclase activators given in Table 3 were found to be maximally inhibitory on O_2^- formation (results not shown). In dibutyryl cyclic AMP-differentiated HL-60 cells, 5'-N-ethylcarboxamido-adenosine inhibited the effects of maximally stimulatory concentrations of fMet-Leu-Phe and UTP by 33 and 47% respectively, whereas the effect of ATP was inhibited by only 15%. Isoproterenol and prostaglandin E_1 inhibited fMet-Leu-Phe- and ATP-induced O_2^- formation by < 10% and UTP-induced O_2^- formation by up to 45%. Pretreatment of the cells with cholera toxin reduced the effects of fMet-Leu-Phe and UTP by up to 70%, whereas the effect of ATP on NADPH oxidase was almost unaffected.

DISCUSSION

As pertussis toxin inhibits the effects of ATP and UTP on NADPH oxidase, and as activation of O_2^- formation exhibits a high degree of specificity for relatively few purine and pyrimidine nucleotides, it is likely that the effects of nucleotides are receptor-mediated. In addition, ATP and UTP activate O_2^- formation at concentrations required to activate other cellular functions presumably occurring *in vivo* (Gordon, 1986). Furthermore, NADPH oxidase activations by ATP, UTP and fMet-Leu-Phe have several characteristics in common, i.e. dependence on extracellular Ca^{2+} , reversibility, requirement for continuous presence of agonist, desensitization, augmentation by cytochalasin B and inhibition by activators of adenylate cyclase.

In agreement with previous reports, we found that ATP and adenosine 5'-O-(3-thiotriphosphate) are potent purinergic agonists in HL-60 cells (Dubyak *et al.*, 1987; Cowen *et al.*, 1988). Interaction of purinoceptors with

pertussis toxin-sensitive G proteins has been documented only for inhibition of adenylate cyclase in rat hepatocytes but not for activation of a purinergic effector system, i.e. phospholipase C (Okajima *et al.*, 1987). Thus, the pertussis toxin sensitivity of NADPH oxidase activation by purinergic agonists is a novel feature of purinoceptors. In addition, the purinoceptors in HL-60 cells show some further properties which are different from those of other purinoceptors. In platelets, ADP is the most potent agonist, and in mast cells only ATP acts as an agonist at purinoceptors (Gordon, 1986). Furthermore, α,β -methylene-ATP and β,γ -methylene-ATP are P_{2X} -purinoceptor agonists but are not activators of NADPH oxidase (Burnstock & Kennedy, 1985). Finally, adenylyl imidodiphosphate is an agonist at P_{2Y} -purinoceptors but not at purinoceptors of HL-60 cells (Rice & Singleton, 1987). These data suggest that the purinoceptors in HL-60 cells do not belong to hitherto characterized purinoceptor subtypes.

In human neutrophils, pertussis toxin and activators of adenylate cyclase differentially inhibit O_2^- formation induced by various classes of hormonal agonists (Lad *et al.*, 1985a; Gryglewski *et al.*, 1987; Verghese *et al.*, 1987; Shirato *et al.*, 1988). NADPH oxidase activation by UTP in HL-60 cells is more sensitive towards inhibition by pertussis toxin and activators of adenylate cyclase than that induced by ATP. These data clearly indicate that UTP does not mediate its effects via purinoceptors. The results strongly support recent data suggesting the existence of pyrimidinoceptors (Kügelen *et al.*, 1987; Häussinger *et al.*, 1987), which, at least in the case of HL-60 cells, interact with pertussis toxin-sensitive G proteins. UTP appears to be the endogenous ligand for this pyrimidinoceptor, as other pyrimidine nucleotides are far less effective activators of O_2^- formation. Activation of NADPH oxidase by pyrimidine nucleotides shows a nucleotide specificity different from that for the activation of other cellular systems, suggesting heterogeneity among pyrimidinoceptors (Shirasawa *et al.*, 1983; Hardebo *et al.*, 1987; Kügelen *et al.*, 1987; Häussinger *et al.*, 1987).

UTP and ATP both potentiate O_2^- formation induced by chemotactic peptides even with both classes of stimuli present at maximally stimulatory concentrations, indicating that these agents activate NADPH oxidase via different receptors. Agonists acting via distinct receptors may additively activate G proteins, and thus may greatly amplify activation of effector systems, i.e. NADPH oxidase and/or phospholipase C. In addition, occupation of purino- and pyrimidinoceptors with agonists may increase the number and/or affinity state of formyl peptide receptors or may prevent their sequestration. The latter possibilities are supported by the finding that cytochalasin B diminishes the potentiating effect of UTP, indicating that both agents act, at least in part, by a mechanism they have in common (Jesaitis *et al.*, 1986).

In contrast to dibutyryl cyclic AMP-differentiated cells, ATP and UTP do not activate NADPH oxidase in dimethyl sulfoxide-differentiated HL-60 cells and in human neutrophils, but they do potentiate O_2^- formation induced by fMet-Leu-Phe. Nucleotides are released into the extracellular space under various physiological and pathological conditions (Goetz *et al.*, 1971; Shirasawa *et al.*, 1983; Gordon, 1986; Forsberg *et al.*, 1987; Hardebo *et al.*, 1987). Thus, ATP and UTP may play roles as intercellular signal molecules in the regulation of O_2^- formation. The physiological function of nucleotides

apparently is to potentiate NADPH oxidase activation rather than to activate the enzyme *per se*. As ATP and UTP *per se* lead to activation of NADPH oxidase in dibutyryl cyclic AMP-differentiated HL-60 cells, possibly due to the expression of very high numbers of purino- and pyrimidinoceptors, these cells provide a useful model system to study purinergic and pyrimidinergetic activation of NADPH oxidase.

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